Supporting Information

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SI Materials and Methods

Generation of IL-7Rα–Floxed Mice. The targeting vector was constructed by Red recombination technology with a murine BAC clone containing the IL-7Rα locus (RP23-267M6). One loxP sequence was inserted 97-bp downstream of IL-7Rα exon 2 and the neomycin resistance gene cassette flanked by Flp recombinase target (FRT) sequences on both sides and one loxP sequence at the 5′ end was inserted 288-bp upstream of exon 2 (Fig. S1*A*). The targeting vector was isolated with the 2.2-kb 5′-homologous fragment, a neomycin resistance gene cassette, and a 5.6-kb 3′-homologous fragment, flanked by diphtheria toxin A subunit cDNA. The linearized targeting vector was introduced into the KY1.1 embryonic stem (ES) cell line. Homologous recombinants were screened by PCR and confirmed by Southern blot analysis with 5′ and 3′ probes (Fig. S1*B*). The neomycin resistance gene cassette was removed from the recombinant allele by infecting targeted ES clones in vitro with the adenovirus expressing Flp recombinase, AxCAFLP (1). IL-7Rα–floxed mice obtained were backcrossed into C57BL/6 mice six to nine times.

Antibodies for Flow Cytometry. The following fluorescent dye- or biotin-conjugated antibodies were used: CD3 (145-2C11), CD4 (GK1.5 or RM4-5), CD8 (53-6.7), TCRβ (H57-597), γδTCR (GL-3), CD11c (N418), CD25 (PC61.5), Qa-2 (69H1-9-9), CD62L (MEL-14), MHC class II (M5/114.15.2), CD19 (MB19-1), BP1 (6C3), IL-7Rα (A7R34), Foxp3 (FJK16s), rat IgG2aκ (eBR2a), hamster IgG isotype control, streptavidin-PE, streptavidin-PE-Cy7, and CaspGLOW TM Fluorescein Active Caspase-3 staining kit (eBioscience); NK1.1 (PK136), CD44 (IM7), GITR (DTA-1), and streptavidin-allophycocyanin (purchased from BioLegend); and CD69 (H1.2F3), cytotoxic T-lymphocyte antigen (CTLA)-4 (UC10-4F10-11), rat IgG2bκ isotype control (A95-1), antimouse Bcl-2 set, and BrdU FITC set (BD Biosciences). Alexa Fluor 488-conjugated anti–Bcl-xL (54H6) and rabbit IgG isotype control were kindly provided by Beckman Coulter (Tokyo). PE-conjugated anti-IgM was purchased from Southern Biotechnology. Allophycocyanin-conjugated CD1d-tetramer loaded with α-GalCer was purchased from ProImmune. The following antibodies were purified from hybridoma supernatant and fluorescent dye- or biotin labeled: heat stable antigen (HSA) (M1/69), B220 (RA3-6B2), CD11b (M1/70), CD5 (53-7.3).

Peripheral T-Cell Isolation and Cell Culture. Lymph nodes (LNs) were isolated from cervical, axillary, brachial, inguinal, and mesenteric regions. Naive T cells were sorted from LNs and spleen as $TCR\beta^+CD25^-NK1.1^-CD44^{low}CD4^+$ or $TCR\beta^+CD44^{low}CD8^+$ cells by FACSAria II. Cells were cultured in RPMI medium 1640 supplemented with 10% (vol/vol) FCS, 50 μM 2-mercaptoethanol, and antibiotics in 5% (vol/vol) CO_2 at 37 °C. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was performed by using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) at OD490 nm.

Adoptive Transfer. Naive CD4 or CD8 T cells from LNs (4×10^5) and spleen (2×10^5) were mixed and labeled with 5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) and then i.v. injected to sex-matched $Rag2^{-/-}$ mice.

Treg Suppression Assay. Naive CD4 and $CD25^+CD4$ T cells were purified by FACSAria II. Naive CD4 T cells (5×10^4) from wild-type mice were seeded in triplicate in 96-well round bottom plate with erythrocyte-removed splenocytes (1×10^5) from $Rag2^{-/-}$ mice (used as antigen-presenting cells) and 0.5 μg/mL of anti-CD3. $CD25^+CD4$ T cells from control or IL-7RcKO mice were added to each well at the indicated ratio. After 72 h culture, proliferation was measured by the MTS assay.

Primer Sequences. The following primer sequences were used:

Perforin-1/For, 5′-CCTATCAGGACCAGTACAAC-3′;
Perforin-1/RV, 5′-GAGATGAGCCTGTGGTAAGC-3′;
GranzymeB/For, 5′-TTTGTGCTGACTGCTGCTCA-3′;
GranzymeB/RV, 5′-TCTAGTCCTCTTGGCCTTAC-3′;
Eomes/For, 5′-GCACCAAACTGAGATGATCA-3′;
Eomes/RV, 5′-TGCATGTTATTGTCCGCTTTGC-3′;
Distal Runx3/For, 5′-AACAGCAGCCAACCAAGTGG-3′;
Distal Runx3/RV, 5′-TGCTCGGGTCTCGTATGAAG-3′;
Distal Runx1/For, 5′-ATGGCTTCAGACAGCATTTTTGAGTCATTT-3′ (2);
Runx1/RV, 5′-ACTGTCATTTTGATGGCTCTATGGTAGGT-3′ (2);
GATA3/For, 5′-TACCGGGTTCGGATGTAAGTC-3′;
GATA3/RV, 5′-CCTTCGCTTGGGCTTGATAAG-3′;
Mcl-1/For, 5′-TCAAAGATGGCGTAACAAACTGG-3′;
Mcl-1/RV, 5′-CCCGTTTCGTCCTTACAAGAAC-3′;
HPRT/For, 5′-GTTGGATACAGGCCAGACTTTGTTG-3′;
HPRT/RV, 5′-GATTCAACTTGCGCTCATCTTAGGC-3′;
pim-1/For, 5′-CACAGTCTACACGGACTTTG-3′;
pim-1/RV, 5′-AGAACACTTGGCCCTTGATG-3′;
$p27^{kip1}$/For, 5′-CTCAGGCAAACTCTGAGGAC-3′; and
$p27^{kip1}$/RV, 5′-TTCGGGGAACCGTCTGAAAC-3′.

1. Kondo S, et al. (2006) Efficient sequential gene regulation via FLP-and Cre-recombinase using adenovirus vector in mammalian cells including mouse ES cells. *Microbiol Immunol* 50(10):831–843.
2. Wong WF, Kurokawa M, Satake M, Kohu K (2011) Down-regulation of Runx1 expression by TCR signal involves an autoregulatory mechanism and contributes to IL-2 production. *J Biol Chem* 286(13):11110–11118.

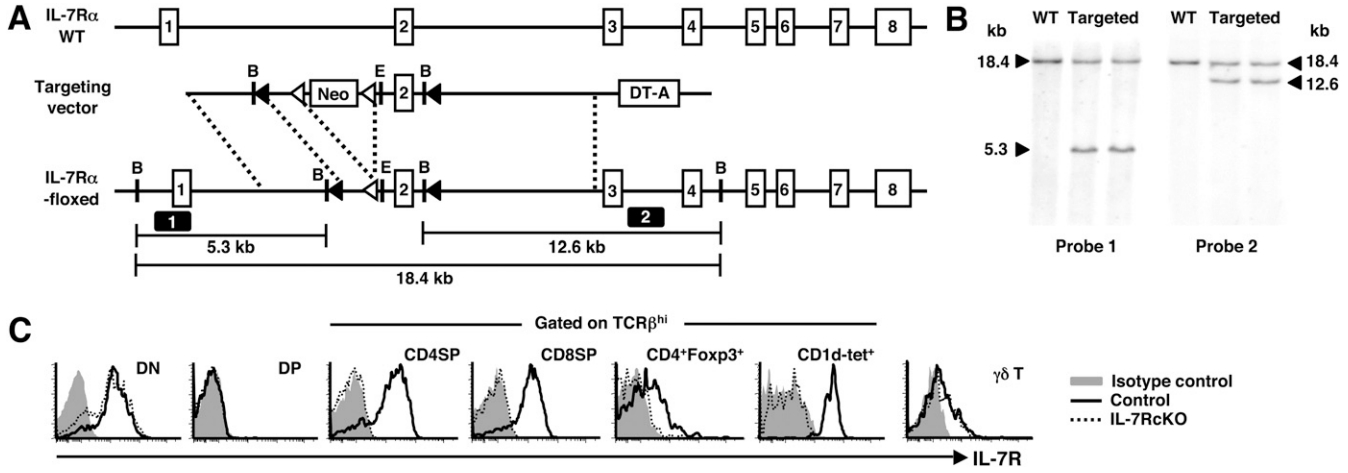

Fig. S1. Conditional targeting of the mouse IL-7Rα locus. (*A*) Strategy for targeting of the mouse IL-7Rα locus. B, BamHI; E, EcoRI; Neo, neomycin resistant cassette; DT-A, diphtheria toxin. Open boxes indicate exons. Open and filled triangles indicate Flp recombinase target (FRT) sequences and loxP sequences, respectively. Numbered filled boxes indicate probes used for Southern blot analysis. (*B*) Genomic DNA from Flp recombinase-treated ES cells were digested with BamHI and analyzed by Southern blotting. Probes used for detection are shown in *A*. (*C*) IL-7R expression in thymocyte subpopulations. Data represent eight independent experiments.

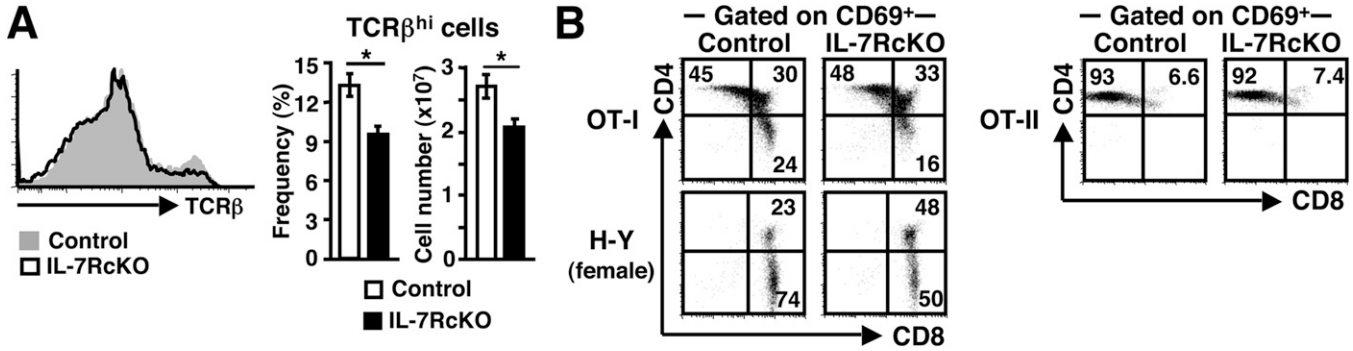

Fig. S2. Positive selection of CD8SP thymocytes is impaired in IL-7RcKO mice. (*A*) TCRβ expression in total thymocytes. Bar graphs show frequency and absolute numbers of $TCR\beta^{hi}$ cells in total thymocytes ($n > 10$). (*B*) CD4 and CD8 expression in $CD69^{+}$ thymocytes from IL-7RcKO or control mice crossed with indicated transgenes on a $Rag2^{-/-}$ background. Data represent three to four independent experiments.

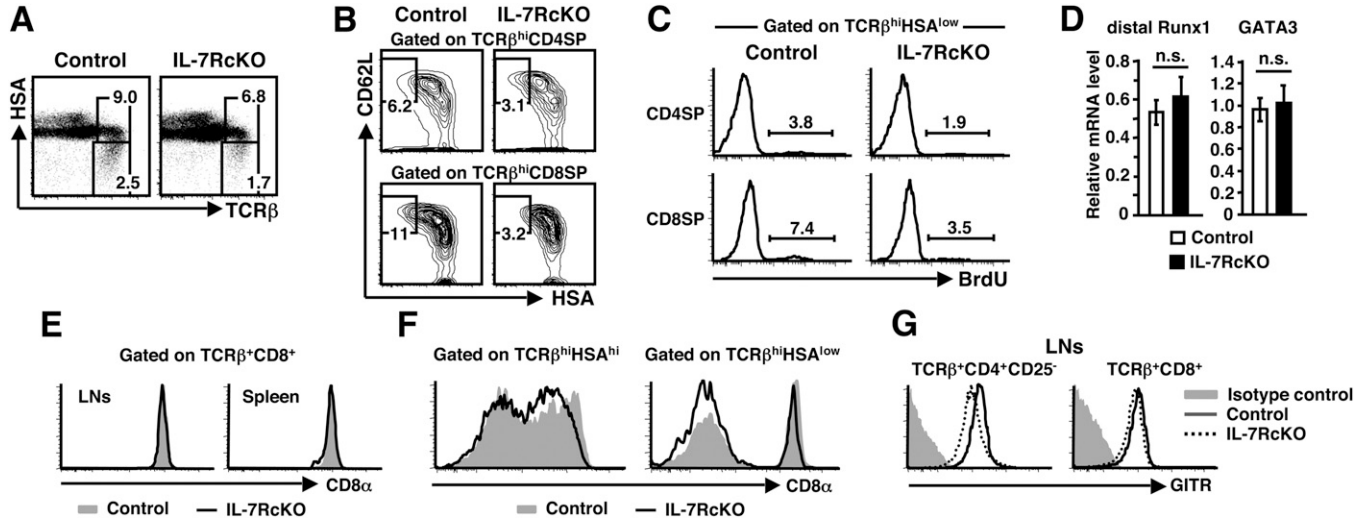

Fig. S3. Mature CD4SP and CD8SP thymocytes are reduced in IL-7RcKO mice. (*A* and *B*) Expression of TCRβ and HSA in total thymocytes (*A*) and HSA and CD62L in TCRβ^{hi}CD4SP and TCRβ^{hi}CD8SP thymocytes (*B*). Data represent five independent experiments. (*C*) BrdU uptake by TCRβ^{hi}HSA^{low} CD4SP and CD8SP thymocytes at 4 h after injection. (*D*) Distal-Runx1 and GATA3 mRNA in TCRβ^{hi}CD4SP thymocytes. Transcripts levels were normalized to HPRT mRNA. Values are the mean ± SEM of triplicate data points from six independent experiments. (*E* and *F*) Expression of CD8α in TCRβ^{+}CD8^{+} cells in LNs and spleen (*E*) and in TCRβ^{hi}HSA^{hi} and TCRβ^{hi}HSA^{low} thymocytes (*F*). Data represent more than 10 experiments. (*G*) Expression of GITR in TCRβ^{+}CD4^{+}CD25^{-} and TCRβ^{+}CD8^{+} cells in LNs. Data represent two independent experiments.

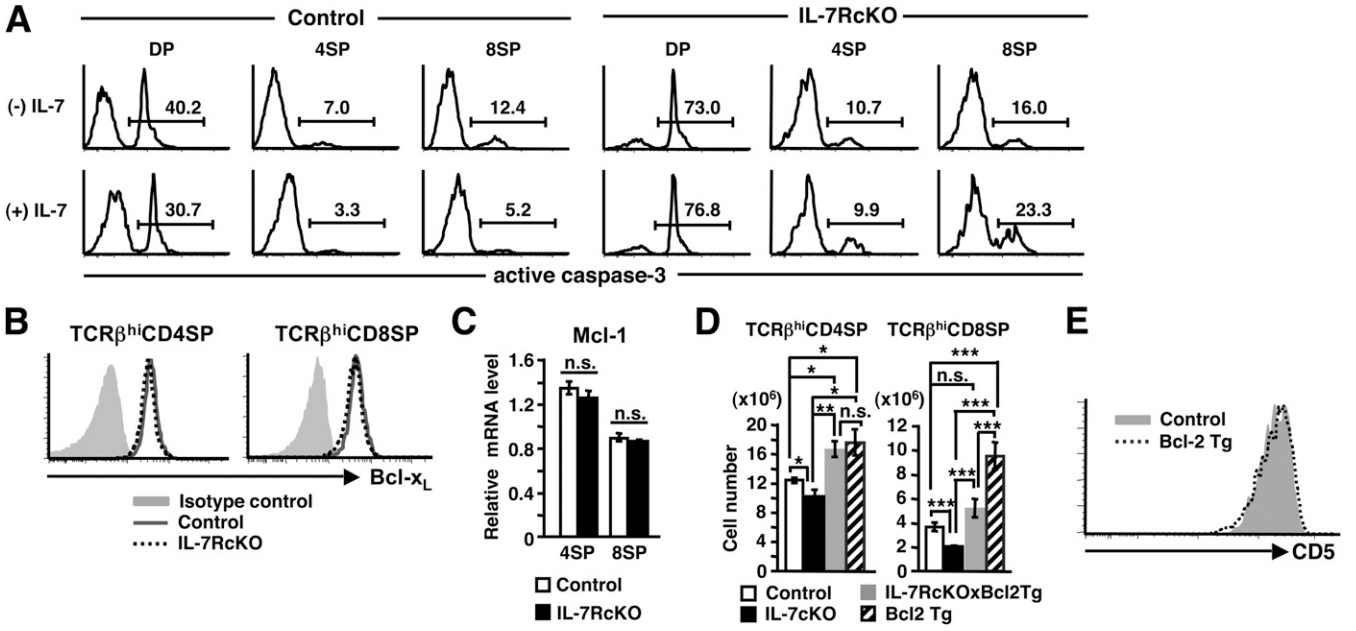

Fig. S4. Expression of antiapoptotic factors in IL-7RcKO thymocytes. (*A*) Total thymocytes were cultured with or without 5 ng/mL IL-7 for 20 h. Active caspase-3 expression in TCRβ^{hi}DP, TCRβ^{hi}4SP, and TCRβ^{hi}8SP thymocytes were shown. Data represent two independent experiments. (*B*) Expression of Bcl-x_L in thymocytes. Data represent two independent experiments. (*C*) Mcl-1 mRNA expression in TCRβ^{hi} SP thymocytes. Values are the mean ± SEM of triplicate data points. Data represent three independent experiments. (*D*) Absolute number of TCRβ^{hi}CD4SP and TCRβ^{hi}CD8SP thymocytes from each genotype (n = 5–11). (*E*) CD5 expression in TCRβ^{hi}CD8SP thymocytes from indicated mice. Data represent three independent experiments.

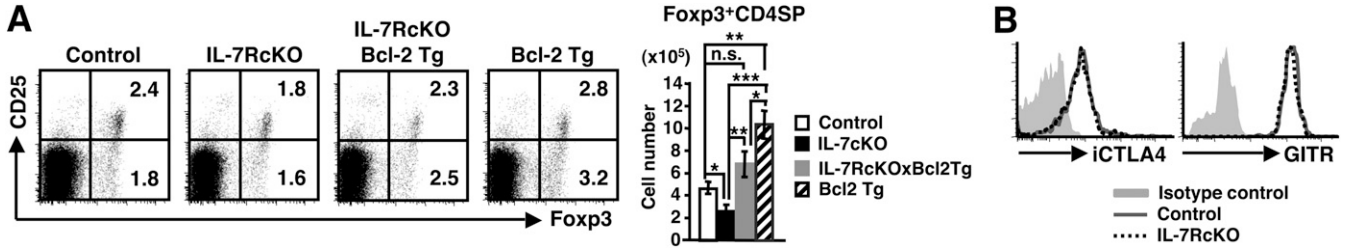

Fig. S5. Effect of Bcl-2 overexpression on Treg development in IL-7RcKO mice. (*A*) Expression of Foxp3 and CD25 in TCRβ^{hi}CD4SP thymocytes from indicated mice (*Left*). Data represent four independent mice of each genotype. Absolute numbers of Foxp3^{+}TCRβ^{hi}CD4SP Tregs from indicated genotypes (n = 4–8) (*Right*). (*B*) GITR and intracellular CTLA-4 expression in Foxp3^{+}TCRβ^{hi}CD4SP Tregs. Data represent three independent experiments.

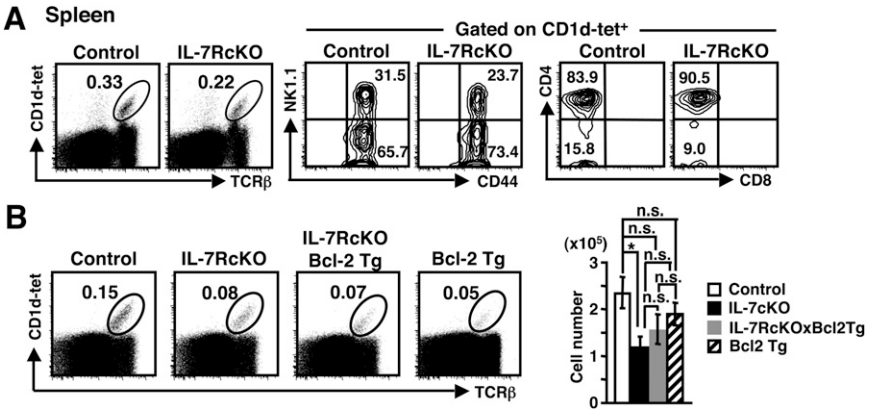

Fig. S6. Bcl-2 overexpression fails to rescue iNKT cell development in IL-7RcKO mice. (*A*) iNKT cells in spleen. Data represent two independent experiments. (*B*) Percentages (*Left*) and absolute number (*Right*) of iNKT cells in thymus from indicated mice (n = 3–6).

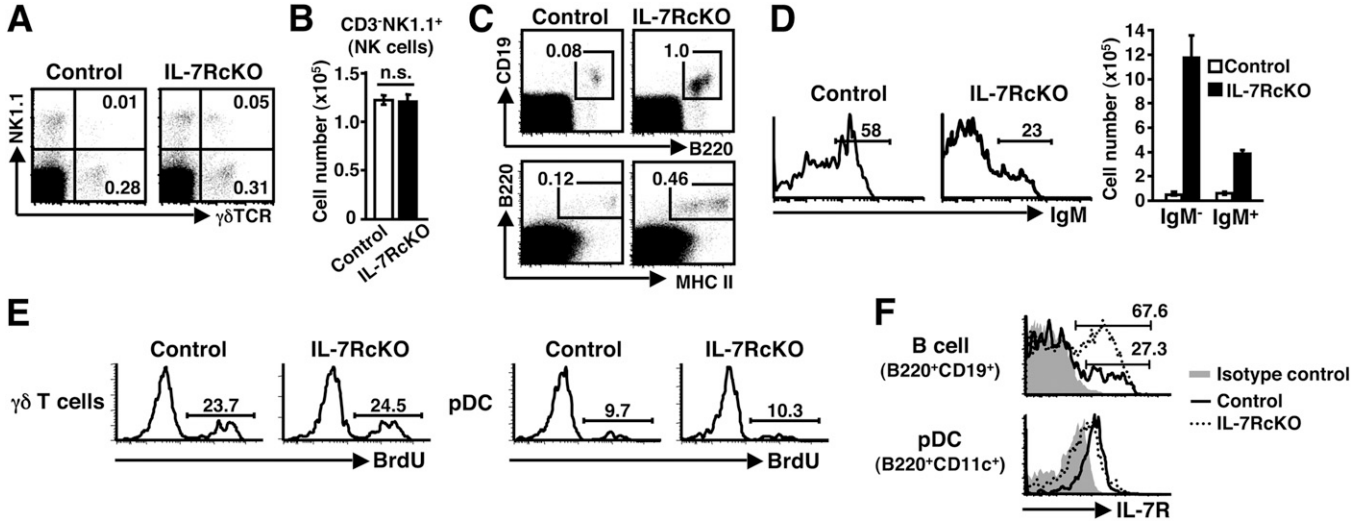

Fig. S7. γδ T cells, B cells, and dendritic cells are increased in IL-7RcKO mice. (*A*) NK1.1^{+}γδ T cells in thymus. Data represent three independent experiments. (*B*) Absolute NK cell numbers in thymus (mean ± SEM, n = 9). (*C*) Expression of CD19, MHC class II, and B220 in thymocytes. Values indicate the percentages of each area. Data represent five independent experiments. (*D*) IgM expression on thymic B220^{+}CD19^{+} cells. Bar graph indicates absolute numbers of IgM^{-} and IgM^{+} thymic B cells (mean ± SEM, n = 5). (*E*) BrdU uptake by γδ T cells and pDCs in thymus at 24 h after injection. Data represent three independent experiments. (*F*) IL-7R expression on thymic B cells and pDCs. Data represent four independent experiments.

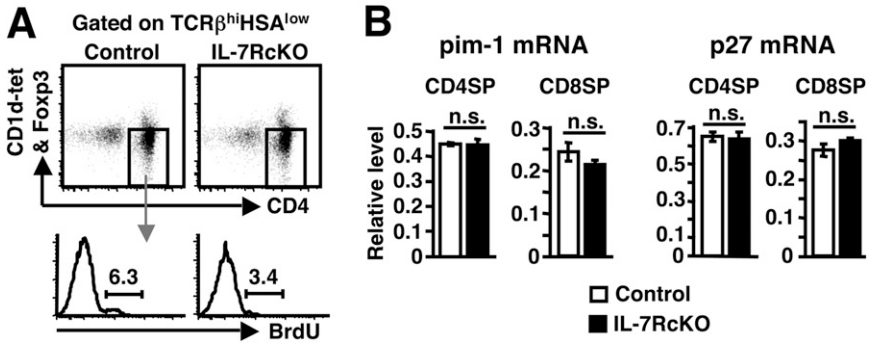

Fig. S8. Proliferation of conventional CD4SP thymocytes is impaired in IL-7RcKO mice. (*A*) BrdU uptake by TCRβ^{hi}HSA^{low} CD4SP thymocytes excluding Foxp3^{+} and CD1d-tet^{+} cells at 24 h after injection. (*B*) Pim-1 and p27^{kip1} mRNA expression in TCRβ^{hi}SP thymocytes. Values are the mean ± SEM of triplicate data points. Data represent two independent experiments.

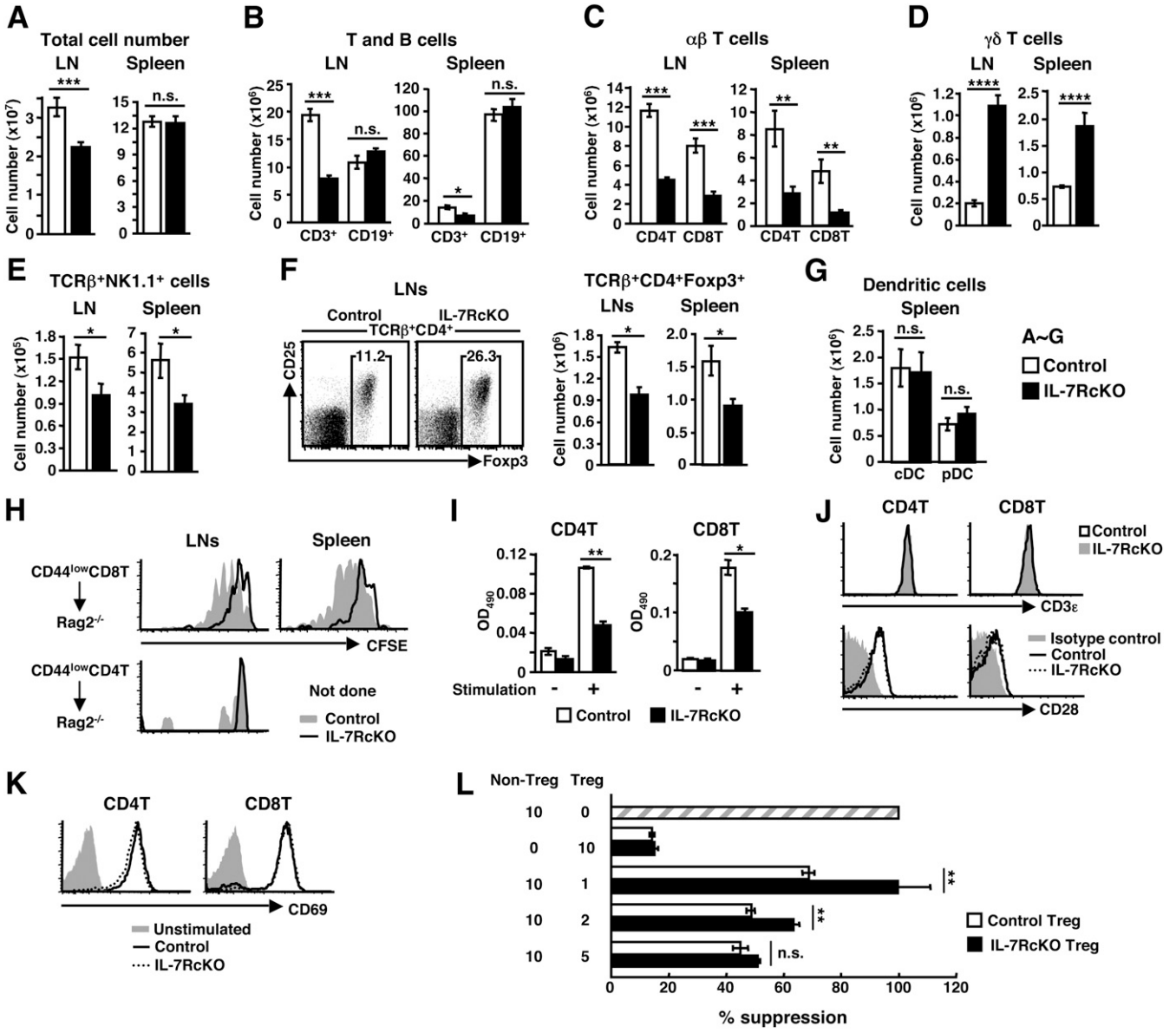

Fig. S9. Reduced numbers and impaired functions of peripheral T cells in IL-7RcKO mice. (*A*) Absolute cell numbers in LNs and spleen ($n = 13$). (*B–E*) Absolute numbers of $CD3^+$ and $CD19^+$ cells ($n = 5$) (*B*), $TCR\beta^+CD4^+$ and $TCR\beta^+CD8^+$ cells ($n = 8$) (*C*), $CD3^+\gamma\delta TCR^+$ cells ($n = 5$) (*D*), and $TCR\beta^+NK1.1^+$ cells ($n = 7$) (*E*), in LNs and spleen. (*F*) Expression of Foxp3 and CD25 in $TCR\beta^+CD4^+$ cells in LNs and absolute number of $Foxp3^+CD4$ Tregs in LNs and spleen ($n = 7$). (*G*) Absolute number of cDCs and pDCs in spleen ($n = 5$). (*A–G*) All mice were analyzed at 4–5 wk old (mean ± SEM). (*H*) Naive CD4 or CD8 T cells were CFSE labeled and transferred to $Rag2^{-/-}$ mice. Five days after transfer, donor populations ($CD3^+CD4^+$ or $CD3^+CD8^+$) in host $Rag2^{-/-}$ mice were analyzed. (*I*) Naive CD4 or CD8 T cells (5×10^4) were cultured in 96-well plate with or without 5 μg/mL of plate-bound anti-CD3ε and 1 μg/mL of soluble anti-CD28. Proliferation was measured by the MTS assay after 72 h. Values are the mean ± SEM of duplicate data points. (*J*) CD3 and CD28 expression of freshly isolated naive T cells from LNs. (*K*) CD69 expression of T cells 18 h after stimulation as described in *I*. (*L*) Treg suppression activity. (*H–K*) All data represent at least two independent experiments.